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Facultative cleaner species in marine temperate waters: the ecological role of juvenile *Diplodus sargus* (Sparidae)

José Nuno de Oliveira Neto

Nº 24378

Thesis supervisor: Prof. Doctor Frederico Almada

Thesis seminar supervisor: Prof. Doctor Emanuel Gonçalves

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1. Introduction

Cleaning symbiosis is an association, during which, an organism defined as "cleaner" removes parasites, dead tissue or unwanted food particles from the epidermis of a co-operating "client" (Galeote & Otero, 1998). This behaviour is found in a variety of terrestrial vertebrates, but is especially common within marine ecosystems. Although they were first noted as being episodic, events of cleaning symbiosis in marine environment are now considered to be a regular occurrence of ecological relevance(Limbaugh, 1961; Grutter, 1999), with over a hundred species of fish already reported as cleaners (Van Tassell *et al.*, 1994; Arnal *et al.*, 2006).

According to their behavior, cleaner fish are classified as facultative or obligatory cleaners. While species from the first group exhibit this behaviour during a specific phase of their life-cycle and rely on other food sources for their diet, fishes on the second group depend mostly on their success obtaining food from these interactions during their entire lifespan (Arnal & Côté, 2000). Due to this and other factors intrinsic to each ecosystem, the frequency and periodicity of cleaning interactions may vary greatly amongst species (Floeter *et al.*, 2007). Besides the relative importance of cleaning interactions as food source and general difference in cleaning rates between obligatory cleaners and facultative ones, obligatory cleaners seem to share some morphological characteristics. Physical traits such as small body size and contrasting striped patterns are common among obligatory cleaners, which may help clients choosing their cleaners through visual cues (Stummer *et al.*, 2004).

Regardless the number of species currently described as cleaners, the ecological relevance of cleaning interactions has been frequently debated (Cheney & Côté, 2005). Difficulties assessing client gain during these interactions, while cleaners benefits remain obvious raised doubts whether these interactions should be consider parasitism, commensalism or mutualism (Cheney & Côté, 2005). Episodes of cheating during cleaning activity, with "cleaners" biting healthy tissue from their clients, resulting in mucus loss, and tissue injuries, have been repeatedly described (Bshary & Schäffer, 2002; Grutter & Bshary, 2003). However clients seem to actively choose the cleaners they interact with in order to avoid these occurrences (Bshary & Schäffer, 2002; Bshary & Grutter, 2005; Pinto *et al.*, 2011). Furthermore, while cleaners tend to be sedentary, defending small territories within which they display this behaviour, many clients have a roaming lifestyle, and visits to these cleaning stations may represent a risk of being preyed upon (Cheney & Côté, 2001; Oates *et al.*, 2012).

In any case, cleaning interactions have an impact on the ecological relationships between clients and cleaners. Several studies indicate that cleaners choose their clients based upon their

parasite loads, and that the size and number of parasites of the clients are directly affected by these interactions (Grutter, 1995; Grutter, 1999; Arnal *et al.*, 2000).

Field experiments have shown that completely removing cleaners from specific reefs, impacts the community within the ecosystem, with several fish opting to roam into other areas (Limbaugh, 1961; Bshary, 2003). This highlights the ecological importance of cleaners as key organisms within their respective communities (but see Grutter, 1996a). However, cleaning behaviour has been frequently studied in tropical fishes and few studies where made concerning species in temperate regions (Limbaugh, 1961; Van Tassell *et al.*, 1994; Galeote & Otero, 1998). Most cleaner species belong to the families Labridae and Gobiidae with a worldwide distribution (Arnal *et al.*, 2006; Baliga & Law, 2016) and there is no trend supporting the assumption that this behaviour is more frequent in tropical waters (Hobson, 1968).

Main cleaner species according to their geographical distribution include, among the Labridae, *Labroides dimidiatus* in the Indic and Pacific Ocean (Grutter, 1997), *Symphodus melops* (Potts, 1973) and *Centrolabrus exoletus* in the north-eastern Atlantic (Henriques & Almada, 1997), and *Centrolabrus melanocercus* in the Mediterranean Sea (Weitzmann & Mercader, 2012; Zander & Sötje, 2002). However, the increase of observations in temperate waters lead to the descriptions of additional facultative cleaners, such as *Coris julis* and *Thalassoma pavo* (Labridae) (Van Tassell *et al.*, 1994) or *Lepadogaster candolii* (Gobiesocidae) (Weitzmann & Mercader, 2012), and obligatory cleaners such as *Elacatinus figaro* (Gobidae) (Sazima *et al.*, 2000)whose behaviour was already known in tropical waters but had not been observed elsewhere (Bertoncini *et al.*, 2009; Narvaez *et al.*, 2015). Additional details are described in the state-of-the-art (Appendix I).

The case of sparids, in general, and *Diplodus sargus*, in particular, represents a striking example that illustrates the strong deficiency regarding field observations in temperate regions. Several species of the *Diplodus* genus (Sparidae) are common along coastal ecosystems of the Mediterranean, Black Sea and the Temperate Atlantic (Rosecchi, 1987), contributing greatly to fish assemblages in rocky infralittoral habitats (Sala & Ballesteros, 1997; Dias *et al.*, 2016). Since sympatric species such as *Diplodus sargus*, *Diplodus vulgaris* and *Diplodus puntazzo* possess a diverse omnivorous diet while co-existing in the same ecosystem, various studies aimed to investigate these species feeding habits, in order to understand, among other factors, potential food partitioning, prey preference and habitat use (Rosecchi, 1987; Sala & Ballesteros, 1997; Figueiredo *et al.*, 2005; Leitão *et al.*, 2007). In these studies, the composition of *D. sargus* diet was greatly explored, analysing quantity, proportion, and recurrence of several food items, proving the species to be highly opportunistic. While examining stomach content of young *D. sargus*, Rosecchi (1987) noted the presence of small ectoparasite copepods (*Caligus pageti*) commonly found on the

epidermis of grey mullets (Mugilidae). This fact was interpreted as occasional cleaning of conspecifics. Other authors also reported the presence of ectoparasites in the stomach contents of *D. sargus* (Mariani, 2001) and another highly similar species, common in the Mediterranean Sea, *D. puntazzo* (Van Tassell *et al.*, 1994).

Behavioural reports of cleaning activity by congeneric sparid fish were only described later for *Diplodus argenteus* (Krajewski, 2007) and recently for *D. sargus* (Abecasis & Abecasis, 2015).

Based on field observations, this work aims to describe the context and frequency of cleaning behaviour by juvenile *D. sargus*. Additionally, the assessment of the ecological relevance of this species as a cleaner and its relative importance for "client" fish is also compared with the information currently available for other temperate cleaner species.

2. Methodology

Field observations were performed along the west coast of Portugal in three marinas in Oeiras (38º 40' 34" N, 9º 19' 05" W), Tróia (38º 29' 36" N, 8º 54' 10" W) and Póvoa de Varzim (41º 22' 08" N, 8º 45' 49" W) from June 2014 to October 2015 (Appendix II).

Scuba-diving observations proved to be ineffective for behavioural observations of juvenile *D. sargus* since the fish always flee in the presence of the observer even in confined areas such as marinas and coastal lagoons. That was probably the main reason why this conspicuous behaviour was only described recently by Abecasis & Abecasis (2015) based on observations from floating peers in marinas. The same methodology was followed in this work. The limitations of this procedure compared to scuba-diving observations include visibility limitations caused by disturbances and reflection on water surface. To minimize these limitations all observations were performed in sheltered areas between floating peers and rocks away from the entrance of the marinas.

Identification of client fish species and visual estimations of the total length of cleaners and clients involved in cleaning events were recorded, given the high concordance between observers (100%) and accuracy of visual size estimations, previously calibrated with plastic stripes with different lengths (<0.5cm). Video recordings were performed during this preliminary work. Cleaners were classified according to their size in five classes with intervals of 2.5cm up to 10cm with one last class including all fish with more than 10cm. Clients, being overall conspicuously larger than cleaners, were classified in five classes with intervals of 10cm up to 40cm with one last class including all fish with more than 40cm. Fish size estimations were only registered when

there was agreement between observers. Observations included group scans in which fish could freely abandon or enter the observation area which comprised a square of approximately 2x2m. A total of 45 observation periods of 60 minutes were performed along the day.

Focal observations of individual fish were also performed in order to analyse the frequency of cleaning behaviour per individual *D. sargus*. These observations were limited to the period in which the focal fish remained within sight of the observers that could follow them along the floating peers. These observations amounted to a total of 105 minutes and included 50 randomly chosen juvenile *D. sargus* followed along the period they remained near the surface (approximately <1m depth) within the observer's sight.

Every occurrence of a cleaning event was registered individually. Cleaning events were registered following Johnson & Ruben (1988). A cleaning event starts with the first physical contact between cleaner and client, as opposed to a simple visual inspection of the client by the cleaner. Cleaning events involve one or several contacts between the cleaner mouth and client fish body (nips) and end with the separation of the pair. For each cleaning event, total duration from the first nip up to the separation of the pair, total number of nips, and client reaction to nips were recorded. Client reaction was considered "indifferent" whenever the swimming pattern of the client remained unchanged after each individual nip, and "negative" whenever the client reaction to contact resulted in a jolt. A visual inspection was reported whenever a juvenile *D. sargus* swam directly into close range of a potential client (less than half the client body length) and no contact was observed between the two. Visual inspections that were not followed by a cleaning event were further described in order to evaluate which fish was responsible for the separation: 1) the client swims away from the cleaner or; 2) the cleaner swims in another direction and shows no further interest in that potential "client". An illustrated description of these interactions can be seen in figure 1.

Interactions requesting cleaning behavior were considered whenever the client's body position conspicuously tilted showing heads-up and heads-down displays in the presence of the cleaner. During such displays client fish halt their normal swimming pattern and position themselves motionless in an angle from 45 to 90º horizontally waiting for the cleaner's interaction. Ambiguous situations where discarded.

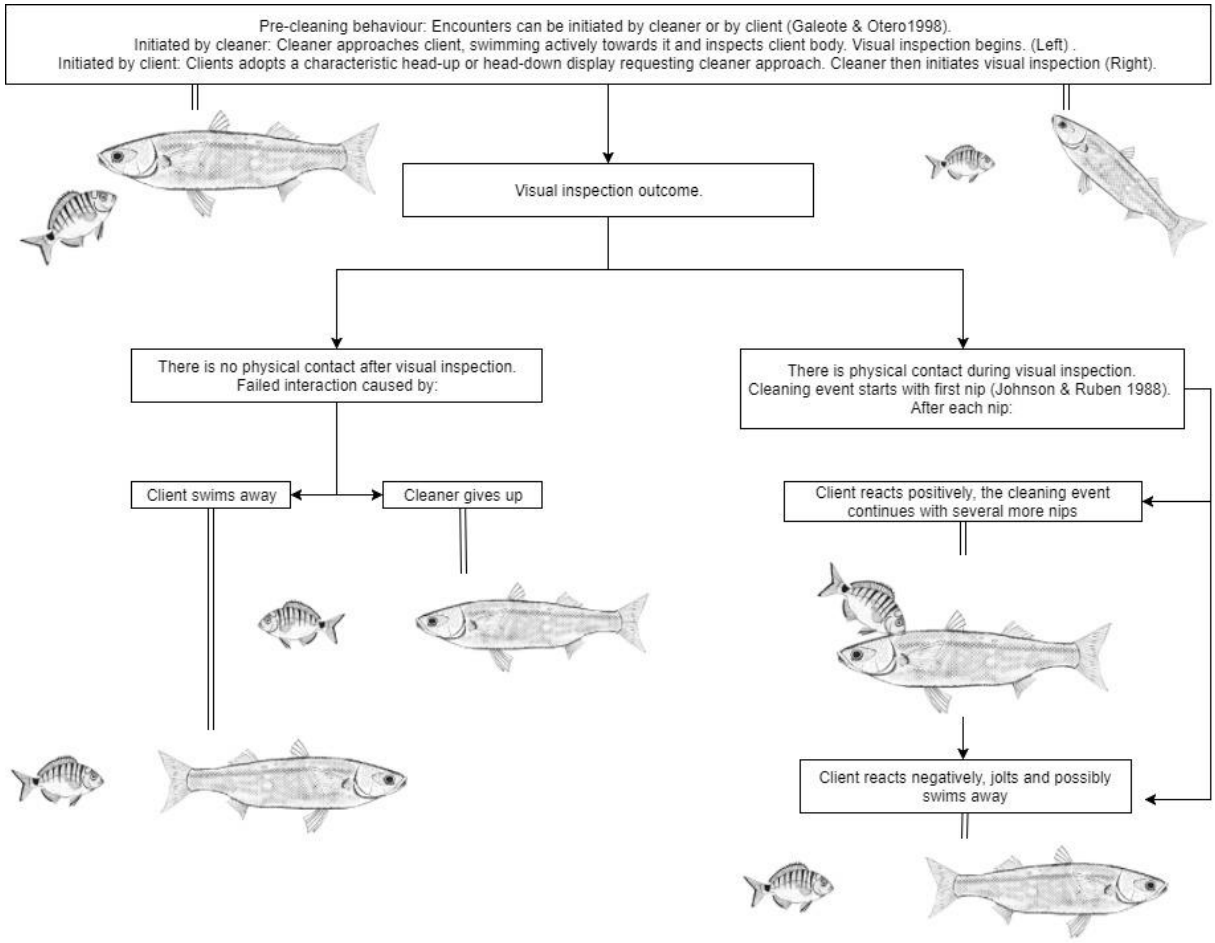

Figure 1. Schematic representation of the cleaner-client interactions between *D.sargus* and its clients.

3. Results

I. Cleaning events, Nips and Duration

During all 45h, a total of 625 cleaning events (M=13.47, SD=7.90) involving 1698 nips (M=37.73, SD=34.73) were observed (for visual demonstration of a nip captured during work see https://www.youtube.com/watch?v=AasslbHYBzA). Cleaning events resulted in a total time of 81.21minutes (M=1.80mn, SD=1.43mn), meaning that we observed cleaning events during 3% of our total observation period. Considering each individual cleaning event, the average number of nips was 2.97 (SD=3.96), and the average duration of interactions was 8.52 seconds (SD=10.90). The observed number of inspections not followed by cleaning events totaled 1058 (M=23.51, SD=26.03) of which 814 (76.93%) were due to avoidance by the potential client and 244 (23.07%) were due to loss of interest from the cleaner *D. sargus*. Considering both cleaning events and unsuccessful interactions, we observed therefore a total of 1683 visual inspections made by *D.sargus* (figure 2).

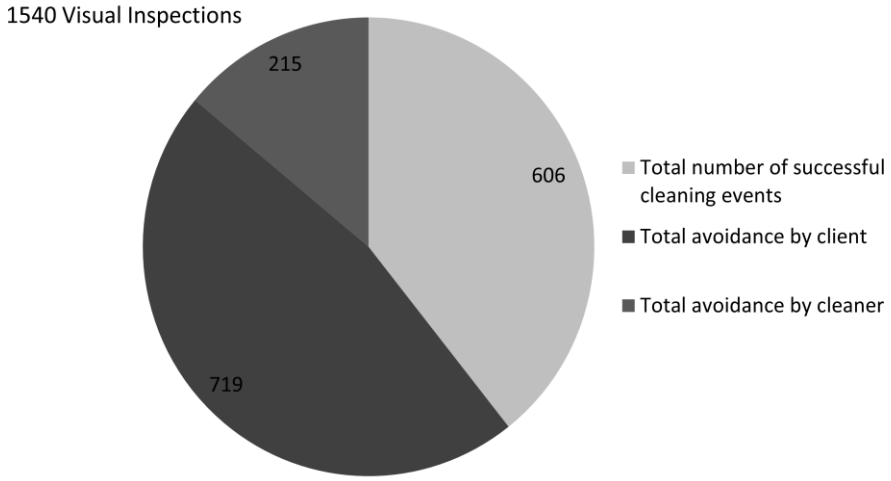

Figure 2. Total number of interactions observed during the 45h of observations between *D.sargus* and its clients. Failed interactions are segregated between those caused by the client avoiding the cleaner or those caused by the cleaner withdrawing.

Cleaning events were observed throughout the year, with the only period where no interactions were observed being a period where no juvenile *D. sargus* were found in march 2015. Relatively to daily patterns, cleaning events were observed during all light-hours, from 07:00 to 20:40 hours. The frequency of cleaning events varied greatly with no statistical evidence of a daily activity pattern (Kruskal-Wallis test: H (10, N= 45) =8,7368 p =,5572). The average cleaning event, average nips and average duration of cleaning events per hour of the day are presented in figure 3.

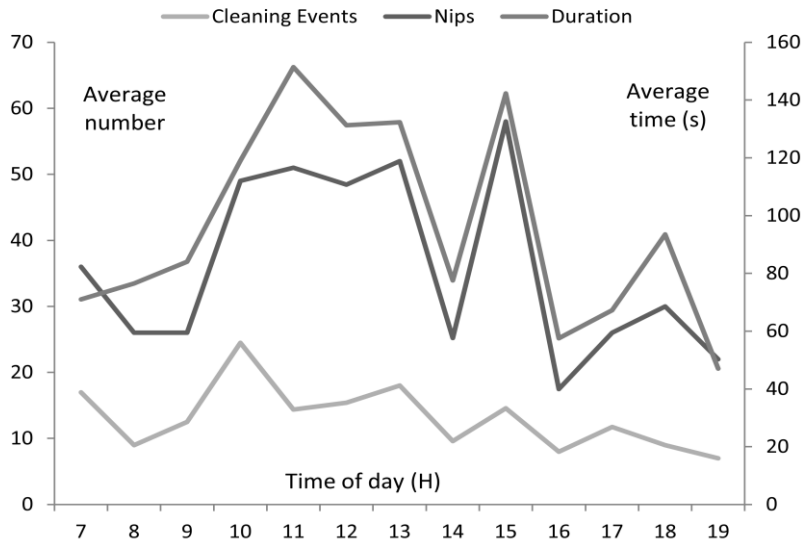

Figure 3. Average number of cleaning events, average number of nips and average duration of cleaning events throughout the day.

As expected, although there was no evidence of activity peaks throughout the day, average nips and average duration of cleaning events followed a very similar pattern. Significant correlation was found between these two measurements (Spearman correlation: $r = 0.789$; $n = 553$, $p < 0.05$), meaning that longer interactions resulted in a relatively linear higher number of nips (figure 4).

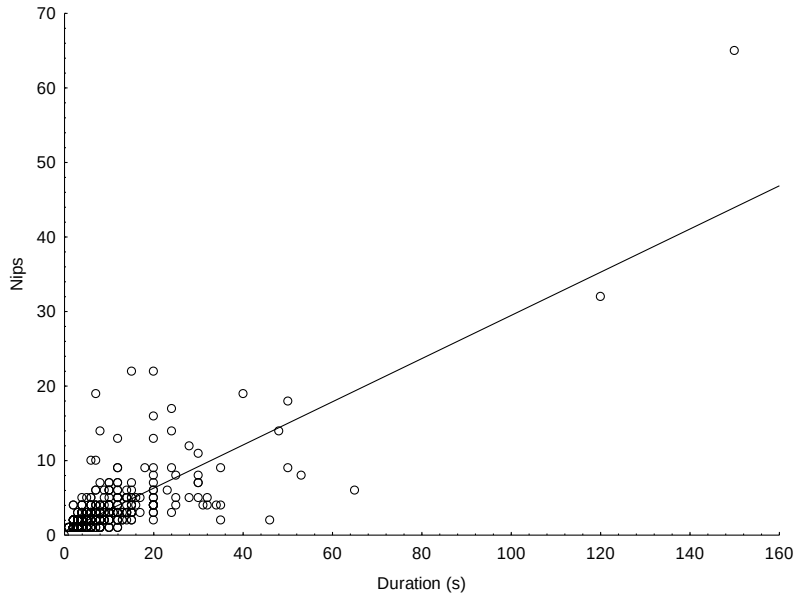

Figure 4. Number of total cleaning event nips per duration of cleaning event.

II. Client fish

D. sargus was observed cleaning multiple different species and the distribution of cleaning events in accordance with client species is shown in table 1.

Table 1. Distribution of cleaning interactions by *D. sargus* according to host species.

Host	Cleaning events	% of total cleaning events	Total client requested interactions
Mugilidae	595	95,20	3
Sarpa salpa	11	1, 76	1
Diplodus sargus	8	1,28	1
Boops boops	4	0,64	0
Seriola sp.	3	0,48	0
Dicentrarchus labrax	2	0,32	0
Symphodus melops	1	0,16	0
Oblada melanura	1	0,16	0
Total	625	-	5

In this table and during observations, cleaning interactions involving only one host and several cleaners were counted only once but situations where one cleaner interacted with several different clients were accounted individually. A large proportion of clients are grey mullets with the most common species along the portuguese marine coast being *Chelon labrosus* and *Liza aurata*. Due to the morphological similarity between different mugilid species these subjects were identified as "Mugilidae" and no further attempt to identify them to the species level was made (Reay & Cornell, 1988).

The reason why Mugilidae represent almost the totality of the fish targeted by juvenile *D. sargus* might be a mere result of the relative abundances of the different host species within the sampled area.

To assess for cleaner recognition from behalf of the client, we tried to consider client behaviour prior to the cleaning interactions. Clients interested in being cleaned typically adopt a posing posture to request interaction by the cleaner (Galeote & Otero, 1998; Stummer et al., 2004). We considered Galeote & Otero (1998) description of heads-up and heads-down display as a reliable estimate of such behaviour and counted each time these displays were made. Since ambiguous observations were excluded from the analysis, the numbers reported here probably represent an underestimation.

III. Client reaction

During cleaning events *D.sargus* clients either reacted positively to physical contact and did not change their previous swimming patterns, or reacted negatively, jolting and swimming away. Of the total physical contacts made between *D.sargus* and client species, clients reacted positively in 1121 occasion (72.5%) (for visual demonstration of positive interaction caught during work see https://www.youtube.com/watch?v=G0RPPqbKmOA) and negatively 525 times (27.5%) (for visual demonstration of a jolt caught during work see https://www.youtube.com/watch?v=NVcBNYkyaY0). Out of these negative reactions, clients terminated the cleaning event 78% of times.

Considering a single client species (using Mugilidae group as example), the probability of negative reactions seems to vary according to cleaner size, and according to client size, with smaller *D. sargus* being more positively received by clients (figure 5), and bigger clients reacting more positively to physical contact with cleaner (figure 6). It is relevant to note that results for *D. sargus* above 10cm are non significant, as only 4 individuals of that group size were observed cleaning.

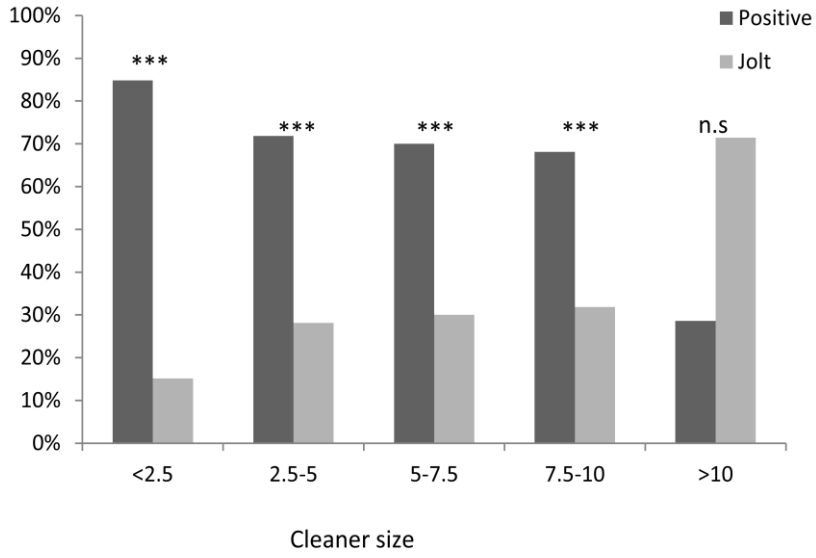

Figure 5. Proportion of positive and negative client reactions to nip according to cleaner size group. Between groups there is a highly significant difference (χ^2 (1, N=550)=25.2531, p<0.001) and within group differences are shown above. Significant at p<0.05*, very significant at p<0.01** or highly significant p<0.001*** or non significant "n.s.".

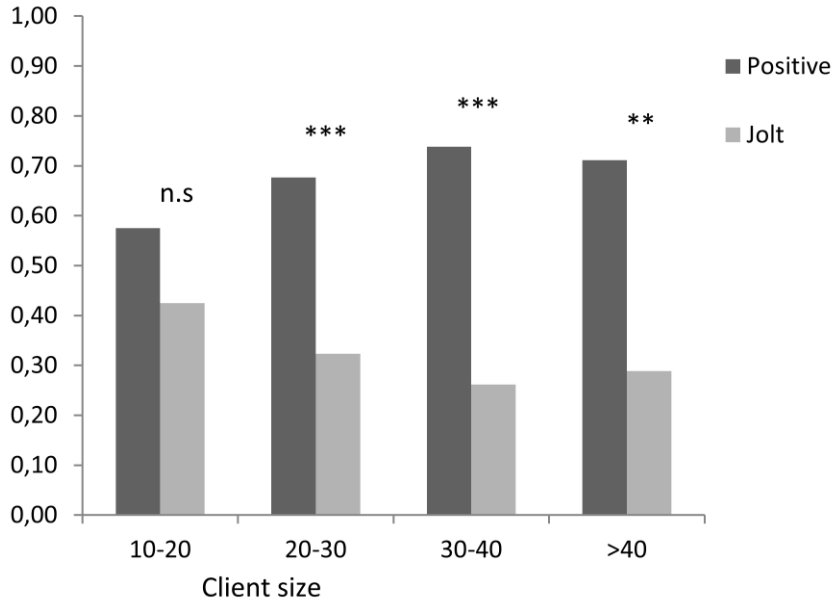

Figure 6. Proportion of positive and negative client reactions to nip according to its own size. Between groups there is a significant difference (χ^2 (1, N=550)=13.8837, p<0.01) and within group differences are shown above. Significant at p<0.05*, very significant at p<0.01** or highly significant p<0.001*** or non significant "n.s.".

There was however no clear correlation between cleaner size and client size, as *D.sargus* of all size groups indifferently cleaned clients of all sizes (Spearman correlation: $r = 0.24$; $n= 553$, $p < 0.05$).

IV. Cleaner preference

Evaluation of specific preferences by cleaner species must account for local abundances of client species. The fact that one particular client species is clearly dominant over other species (i.e. almost all cleaning events are directed to one particular species) could be explained by: 1) there is a bias by the cleaner fish to preferentially clean one particular species; or 2) the client species is the most abundant one and, instead of a preference by the cleaner fish, there is another variable to consider, which is the relative availability of client species. In this last case, the cleaner is opportunistic and targets all species with an appropriate body size and swimming behaviour according to their abundances.

To evaluate a possible bias by juvenile *D. sargus*, seven visual census were conducted to obtain a general idea of the local ichthyofauna and relative species abundances (table 2). It is relevant to note that Mugilidae represent the most abundant group although the abundances of small *Boops boops* and *Atherina presbyter* were relatively high. *Sarpa salpa* shows the opposite pattern, while their abundance was low during all census, they were the second most common species to be cleaned by *D. sargus*. Overall, these results point to an inclusive interpretation: the abundance of client species is important, but it is also important to evaluate if the client fish have an appropriate size (larger than the cleaner fish) and display appropriate behaviour (resting motionless or slow swimming).

Table 2. Total number of individuals counted during each of the visual census. The census were made: **1**. 04/07/2014, **2.** 17/09/2014, **3.** 25/10/2014, **4.** 06/03/2015, **5.** 24/06/2015, **6.** 22/07/2015 and **7.** 27/09/2015. Information regarding *Muraena Helena, Gobius cobitis, Mullus surmuletus, Diplodus cervinus* and *Diplodus puntazzo* is not shown here due to their low recurrence and the fact they were not observed interacting with *D. sargus*.

	Visual census								
Species	**1st**	**2nd**	**3rd**	**4th**	**5th**	**6th**	**7th**	**Total**	**Relative abundance**
Mugilidae	2200	715	390	180	360	520	296	4661	0,35
Diplodus sargus	190	152	230	11	330	610	605	2128	0,16
Diplodus vulgaris	40	5			126	76	16	263	0,02
Oblada melanura	50					10	1	61	0,00
Sarpa salpa		85			25	8	4	122	0,01
Boops boops		130	410	160	420	110	2145	3375	0,25

Dicentrarchus labrax	4	4	1		1	8	19	37	0,00
Atherina presbyter		380	650	130	780	380	520	2840	0,21
Symphodus melops		2			21			23	0,00
Total	2484	1473	1681	481	2063	1722	3606	13510	

During cleaning events, *D. sargus* did not clean all parts of their clients equally. We divided clients in 3 body sections; 1)Head, starting from the tip of the snout down to the base of the pectoral fin; 2)Flank, starting from the pectoral fin to the base of the anal fin; 3) Tail, beginning at the anal fin and ending at the tip of the tail. We tried to register which part *D. sargus* attempted to clean during each nip. While total observed nips for each of these body sections was overall equivalent (591 nips Head, 535 nips Flank, 572 nips Tail), between cleaner group sizes there seemed to be a difference concerning preferred area χ^2 (1, N=550)=59.1509, p<<0.001). When we look at the proportions, we can see that within smaller *D. sargus* there appears to be no clear preference of clients body section, while for bigger groups the head seems to be the preferred part of the body (figure 7).

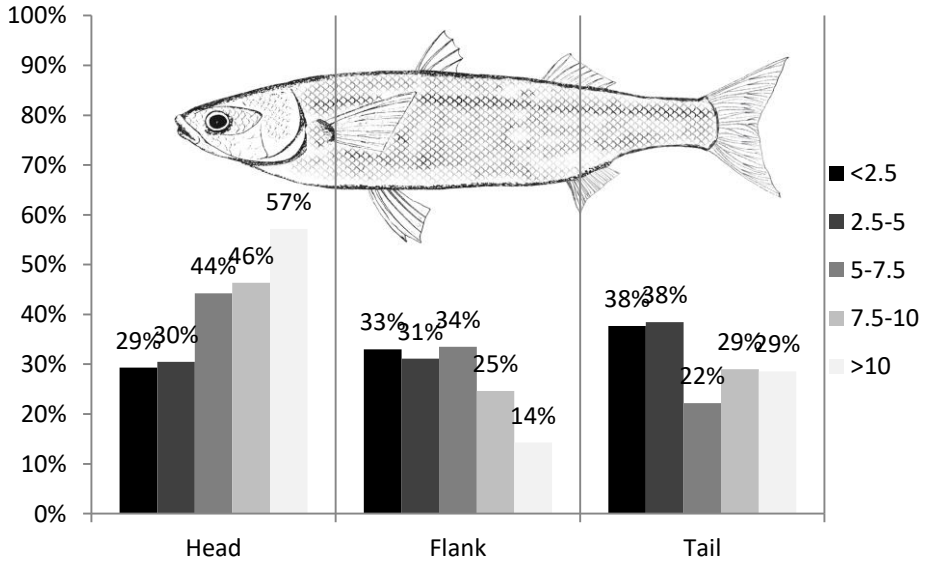

Figure 7. Proportion of nips directed to each of the clients body sections within each of the 5 *D. sargus* size groups.Within groups

V-**Focal observations**

Out of the 50 followed individuals, 21 were seen visually inspecting potential clients, resulting in a total of 23 cleaning events and 44 failed interactions. During cleaning events 34 physical contacts between client and cleaner were made (M=1.47, SD=2,18), and cleaning events

amounted for a total of 189 seconds (M=8.27s, SD=13,14), meaning that *D. sargus* cleaned during 3% of the time they were followed.

Of the 23 cleaning events, 21 were directed to Mugilidae, while the remaining two were made to another *D. sargus* and a *Sarpa salpa*. Concerning failed interactions, most were due by avoidance of the cleaner, with the exception of two of them which were directed to *Dicentrarchus labrax* and *Seriola* sp., in both cases the cleaner swam away after visual inspection.

4. Discussion

I-Overall aspects of cleaning by *D. sargus*

Although *D.sargus* cleaning had been previously hypothesized (Rosecchi, 1987; Mariani, 2001) and recently reported (Abecasis & Costa Abecasis, 2015), the frequency of cleaning events and its ecological relevance was yet to be described.

Residual cleaning activity by individuals above 10cm (4 individuals observed with a total of 7 nips) and absence of cleaning activity from individuals above 15cm, had already been hypothesized by Rosecchi (1987), through the observation of gut contents. Cleaning activity in *D. sargus* is therefore limited to juvenile individuals and should only be observable as long as there are *D. sargus* belonging to the age groups of up to 1 year (Gordoa & Molí, 1997), which coincide with the 10cm total length threshold. Considering that 1 year old juveniles (with approximately 10cm total length) can be replaced by a new cohort of post-larvae due to the onset of a new settlement phase, cleaning events could be observed all year long, which is reported for the first time in this work. Considering the first cohort (>10cm), it is important to note that they move off-shore towards deeper subtidal waters during the end of their first summer as water temperature cool down (Rosecchi, 1987). With this dynamics in mind there is a possibility, that harbours and coastal lagoons create a perfect and unique micro-environment to allow this year-long situation. Furthermore, *D. sargus* present an omnivorous and possibly opportunistic feeding regimeopening the possibility that sheltered shallow waters offer the optimal conditions for this type of foraging (Sala & Ballesteros, 1997; Figueiredo *et al.*, 2005; Leitão *et al.*, 2007).

Concerning *D. sargus* clients several factors have to be considered. . Besides the species belonging to the family Mugilidae, which were the most abundant ones in the study area, there was no direct relation between client species abundance and frequency of interactions. While some reports have found a relation between these two factors (Galeote & Otero, 1998; Arnal *et*

al., 2000), other factors may also explain the difference in interactions we observed among potential client species. In both Grutter (1995) and Arnal (2000), cleaner selectivity of potential clients is reported according to several characteristics of the client. One of them, the size of the client fish species, could explain the results reported above. Bigger clients are usually more attractive to cleaners for two main reasons: first because size is considered a strong correlate with parasite load (Grutter, 1995) and second, because bigger clients may possess richer mucus (Arnal & Morand, 2001b), two characteristics that may attract cleaners as they represent better food sources. While size and abundance of species are usually negatively correlated, within the study area, Mugilidae were both the most abundant and the largest species. The combined effect of high abundance and larger body size could help to explain the high frequency of interactions of this cleaner species with Mugilidae (Floeter *et al.*, 2007). On the other hand, the high abundance of *Boops boops* and *Atherina presbyter* was a consequence of the presence within the study area of big schools of juveniles less than 10cm total length. These small fish were never approached by *D. sargus* and usually avoided all other fish species. As clients are usually 1.5x larger than their cleaners (Sazima *et al.*, 2000), this would exclude these schools of fishes as potential clients.

Client behavior might also be an important factor to determine both cleaning frequency and interaction outcome with different species (Arnal & Côté, 2000; Zander & Sötje, 2002; Soares *et al.*, 2008). Although failed interactions were mostly due to client swimming away from the inspecting cleaner (77%), some clients did actively request cleaning interaction through specific body postures and reacted positively to cleaner nips. The low numbers of *Sarpa salpa* contrasted with the fact that the rare heads-up and heads-down display observed in the Mugilidae was observed once in this species. Furthermore, while in general the proportion of positive and negative reactions to nips was of 3:1, for *Sarpa salpa* we accounted for a total of 116 positive reactions only 2 jolts, which in both cases terminated the interaction. One particular cleaning event was interesting as *S. salpa* interacted with a single juvenile of *D. sargus* in a cleaning event that lasted for 150 seconds and during which over 65 contacts were made, none of which *S. salpa* reacted negatively to. It is important to underline that *S. salpa* is known to interact with other cleaners with which he also often solicits interaction (Henriques & Almada, 1997; Sabatino *et al.*, 2007), therefore *S. salpa* does possess the required mechanisms to recognize and interact with cleaners. It is interesting to note that *D. sargus* juveniles approached and cleaned two potential predators: *Dicentrarchus labrax* and *Seriola* sp. with the latter being also observed actively chasing and eating small *D. sargus* within the study area. Cleaners generally approach potential predators with caution and predators are very rarely cheated upon as they may cheat in return by eating the

cleaner (R. Bshary, 2002; Barbu *et al.,* 2011). This shows that the ecological niche of clients was not a determining factor for the existence of interactions.

In other hand, client behavior might also explain intraspecies differences and why, within Mugilidae of different size groups, the number of positive and negative reactions was different. Mugilidae of smaller sizes reacted more often negatively to the approach and to nips from *Diplodus sargus,* while bigger jolted less often after *D. sargus* nips.

Three hypotheses can be formulated to explain these differences: Client learning process (Sabatino *et al.*, 2007) could start with unwilling individuals, resulting in "hit and run" interactions (Johnson & Ruben, 1988) and lead these fish to get accustomed to the presence of cleaners, with larger and less naïve Mugilidae would be more willing to interact.

2) Higher tolerance for pain since it is possible that *D. sargus* may cheat on various occasions (Pinto *et al.*, 2011).

3) 3) Size difference between client and cleaner could explain the differences between the behaviour of smaller and larger Mugilidae. As smaller cleaners are also less likely to be negatively received by clients than bigger ones, it is possible that stress response to the approach of other species may lower with higher size differences.

Regarding behavior there may also be a learning process of *D. sargus* in regard to how to interact with clients. There is a possibility that smaller naïve *D. sargus* clean all parts of the client body indifferently, while larger more experience *D. sargus* slowly gain a preference for the head, gills and base of the pectoral fins. As *Caligus* were the ectoparasites reported in *D. sargus* gut contents (Rosecchi, 1987; Mariani, 2001), and they show preference for the head of their hosts (Alaş & Öktener, 2017), this choice might be related with higher parasite load in the frontal zone of the client.

Rosecchi (1987) reported parasitic Caligidae in 10% of the analyzed *D. sargus* stomach contents while Mariani (2001)reported *a* occurrences 17,9%. In this work focal observations of the individual *D. sargus* followed revealed that 24% (12 in 50) engaged in cleaning events. However, if we assume that all jolts were cheating occurrences without removal of parasites and remove these from our analysis, only 9 (18%) of the cleaners were observed removing parasites, a result that is very similar to that reported by Mariani (2001). A similar approach to the one used by Rosecchi (1987) and Mariani (2001), aiming for the stomach contents of juvenile *D. sargus* could help to confirm these results.

II- *D. sargus* as a temperate facultative cleaner

As we proved that *D. sargus* engages in cleaning interactions with several client species, and as we try to understand the frequency and overall relevance of such interactions, it is interesting to try to compare our data (table 5) with that of other works regarding cleaners in temperate water; Henriques & Almada (1997) for *Centrolabrus exoletus* in the Atlantic (table 6) and Sabatino *et al.* (2007) for *Symphodus melanocercus* in the Mediterranean (table 7), these two Labridae are considered to be two of the most prominent cleaners within their respective range.

While our work totaled 45h, Henriques & Almada completed 50h and Sabatino *et al.* 32h only, despite this difference in hours we can however make some assumptions regarding the differences. When compared to the other two species, *D. sargus* had a higher number of cleaning events per hour (13.2 *D. sargus,* 5.08 *C. exoletus* and 4.75 *S. melanocercus*), but with a lower number of species (8 *D.s*, 12 *C.e* and 19 *S.m*). Furthermore while Mugilidae accounted for 95.45% of all interactions with *D. sargus,* the most frequent client of *C. exoletus* was *S. melops*, which accounted for 49.61% of interactions. The most frequent clients of *S. melanocercus* was *Symphodus tinca* with 23,03% of total interactions. It is relevant to note that the most frequent clients of both *C. exoletus* and *S. melanocercus*, also belong to the family Labridae, a family with numerous species known as cleaners in their respective habitats. This ultimately could facilitate these species ability to recognize and interact with other cleaners.

Table 3. Cleaning interactions by juvenile *D. sargus*, proportion of interactions and total frequency of interactions during the observation period (45h).

D. sargus (this work)

Host Family	Host species	Cleaning interactions	Rank	% of total interactions	Frequency (per hour)
Mugilidae	Unidentified	630	1	95,45	13,13
Apogonidae	*Apogon imberbis*				
Sparidae	*Boops boops*	4	4	0,61	0,08
	Diplodus annularis				
	Diplodus puntazzo				
	Diplodus sargus	8	3	1,21	0,17
	Diplodus vulgaris				
	Oblada melanura	1	7.5	0,15	0,02
	Sarpa salpa	11	2	1,67	0,23
	Spondyliosoma cantharus				
Pomacentridae	*Chromis chromis*				
Labridae	*Coris julis*				
	Ctenolabrus rupestris				
	Labrus bergylta				
	Labrus viridis				
	Symphodus cinereus				
	Symphodus mediterraneus				
	Symphodus melops	1	7.5	0,15	0,02
	Symphodus ocellatus				
	Symphodus roissali				
	Symphodus rostratus				
	Symphodus tinca				
Moronidae	*Dicentrarchus labrax*	2	6	0,30	0,04
Mullidae	*Mullus surmuletus*				
Carangidae	*Seriola sp.*	3	5	0,45	0,06
Serranidae	*Serranus cabrilla*				
	Serranus scriba				
Molidae	*Mola mola*				
	TOTAL	660			13,75

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Table 4. Cleaning interactions by *C. exoletus*, proportion of interactions and total frequency of interactions during the observation period (50h).

	***Centrolabrus exoletus* (Henriques & Almada, 1997)**				
Host Family	**Host species**	**Cleaning interactions**	**Rank**	**% of total interactions**	**Frequency (per hour)**
Mugilidae	**Unidentified**	2	8	0,79	0,04
Apogonidae	***Apogon imberbis***				
Sparidae	***Boops boops***				
	Diplodus annularis				
	Diplodus puntazzo				
	Diplodus sargus	2	8	0,79	0,04
	Diplodus vulgaris	9	5	3,54	0,18
	Oblada melanura				
	Sarpa salpa	3	6	1,18	0,06
	Spondyliosoma cantharus	1	11	0,39	0,02
Pomacentridae	***Chromis chromis***				
Labridae	***Coris julis***	**15**	**3**	**5,91**	**0,30**
	Ctenolabrus rupestris	1	11	0,39	0,02
	Labrus bergylta	**82**	**2**	**32,28**	**1,64**
	Labrus viridis				
	Symphodus cinereus				
	Symphodus mediterraneus				
	Symphodus melops	**126**	**1**	**49,61**	**2,52**
	Symphodus ocellatus				
	Symphodus roissali	10	4	3,94	0,20
	Symphodus rostratus	2	8	0,79	0,04
	Symphodus tinca				
Moronidae	***Dicentrarchus labrax***				
Mullidae	***Mullus surmuletus***				
Carangidae	***Seriola sp.***				
Serranidae	***Serranus cabrilla***				
	Serranus scriba				
Molidae	***Mola mola***	1	11	0,39	0,02
	TOTAL	254			5,08

Table 5. Cleaning interactions by *S. melanocercus*, proportion of interactions and total frequency of interactions during the observation period (32h).

***Symphodus melanocercus* (Sabatino et al.2007)**

Host Family	Host species	Cleaning interactions	Rank	% of total interactions	Frequency (per hour)
Mugilidae	Unidentified				
Apogonidae	*Apogon imberbis*	1	18	0,66	0,03
Sparidae	*Boops boops*				
	Diplodus annularis	5	7.5	3,29	0,16
	Diplodus puntazzo	3	12	1,97	0,09
	Diplodus sargus	5	7.5	3,29	0,16
	Diplodus vulgaris	2	15	1,32	0,06
	Oblada melanura	1	18	0,66	0,03
	Sarpa salpa	**21**	**3**	**13,82**	**0,66**
	Spondyliosoma cantharus				
Pomacentridae	*Chromis chromis*	2	15	1,32	0,06
Labridae	*Coris julis*	15	4	9,87	0,47
	Ctenolabrus rupestris				
	Labrus bergylta				
	Labrus viridis	4	9.5	2,63	0,13
	Symphodus cinereus	3	12	1,97	0,09
	Symphodus mediterraneus	4	9.5	2,63	0,13
	Symphodus melops				
	Symphodus ocellatus	2	15	1,32	0,06
	Symphodus roissali	6	6	3,95	0,19
	Symphodus rostratus	3	12	1,97	0,09
	Symphodus tinca	**35**	**1**	**23,03**	**1,09**
Moronidae	*Dicentrarchus labrax*				
Mullidae	*Mullus surmuletus*	1	18	0,66	0,03
Carangidae	*Seriola sp.*				
Serranidae	*Serranus cabrilla*	10	5	6,58	0,31
	Serranus scriba	**29**	**2**	**19,08**	**0,91**
Molidae	*Mola mola*				
	TOTAL	152			4,75

Considering the frequency of cleaning interactions for each species, *D. sargus* displayed aproximately 13.75 cleaning events per hour, *C. exoletus* 5.08 cleaning events per hour and *S. melanocercus* 4.75 cleaning events per hour. Although through this analysis *D. sargus* seems to be a more active cleaner, one must consider that the methodology was not identical in these three species. Labrid data was collected while scuba-diving which can ultimately result in large

differences in information accuracy. Furthermore, during our group scans we must consider the high desnsities of juvenile *D. sargus* as compared to *C. exoletus* which is mostly solitary and territorial. In contrast *D. sargus* formed schools of up to 40 individuals. Nevertheless, the high abundances of juvenile *D. sargus* do not affect the ecological relevance of the cleaning behaviour they display toward other species, highlighting even more the overall effect of the cleaning rate/hour described above.

However, Henriques & Almada (1997) provide information concerning frequency through focal observations of *C. exoletus* and Arnal & Morand (2001a) discuss individual frequencies of *S. melanocercus*. Individual focal observations of *C. exoletus*, resulted in 8.4 cleaning events per hour and an estimated investment of 7% of the time of each fish spent in cleaning interactions, compared with juvenile *D. sargus* 13.14 cleaning events per hour and 3% of the time invested in cleaning interactions. In other words *D. sargus* interacts more often but spend less time cleaning other fish compared to *C. exoletus*. In the case of *S. melanocercus* however, the number of interactions per hour reached 152, and individual fish spent up to 16.8% of their time involved in cleaning interactions, a result far higher than that previously calculated and frequencies far superior to those of the other two species.

In table 6, we compare cleaning frequencies of several known facultative cleaners from temperate waters, and further compare these rates with two obligate tropical cleaners *Labroides dimidiatus* and *Gobiosoma evelynae*. We compile results from both focal observations and estimated frequency obtained through local scans.

Table 6. Calculated cleaning frequencies of several cleaners observed through focal observations and calculated through local scans

Species	Obligate or Facultative	Number of cleaning events per hour	Reference
Elacatinus evelynae	Obligate	266 (focal observation)	(Whiteman & Côté, 2002)
Labroides dimidiatus	Obligate	228 (focal observation)	(Grutter, 1996b)
Symphodus melanocercus	Obligate?	152 (focal observation)	(Arnal & Morand, 2001a)
Elacatinus evelynae	Obligate	28,45 (local scans)	(Johnson & Ruben, 1988)
Thalassoma pavo	Facultative	24.8 (focal observation)	(Narvaez *et al.*, 2015)
Coris julis	Facultative	21.4 (focal observation)	(Narvaez *et al.*, 2015)
Diplodus sargus	Facultative	13,75 (local scans)	This study
Diplodus sargus	Facultative	13,14 (focal observation)	This study

Centrolabrus exoletus	Facultative	8,4 (focal observation)	(Henriques & Almada, 1997)
Centrolabrus exoletus	Facultative	5,08 (local scans)	(Henriques & Almada, 1997)
Symphodus melanocercus	Facultative	4,75 (local scans)	(Galeote & Otero, 1998)

Ultimately we may confirm that juvenile *D. sargus* engage in cleaning interactions at a rate that may be comparable to some of the already known facultative cleaners of temperate waters. While it is impossible to determine, for now, which are the most influential characteristics which affect cleaning frequencies, it would appear that, such as with their feeding regime, *D. sargus* are opportunistic and target species which are most abundant and prone to this type interactions.

In a worldwide framework individual frequencies and cleaning events by juvenile *D. sargus* duration tend to be low. However, compared to the northeastern Atlantic and Mediterranean ichthyofauna these marks are unexpectedly high, considering that this species was only recently observed to clean other fish and that *C. exoletus* is usually considered to be the main cleaner species along the Portuguese coast. Furthermore the high number of juvenile *D. sargus* present in harbors and coastal lagoons during settlement, where these fish reach high abundances and densities, to the point that such coastal areas are classified as nurseries (Dias *et al.*, 2016), highlight even more their ecological relevance in temperate waters. Additional studies are necessary to compare the compared measurements stated above, and to understand the importance of this species within its area of distribution.

5. References

Abecasis, D., & Costa Abecasis, A. R. (2015). First report of cleaning behaviour in white sea bream (Diplodus sargus). *Marine and Freshwater Behaviour and Physiology*, *48*(1), 71–75. http://doi.org/10.1080/10236244.2014.990700

Alaş, A., & Öktener, A. (2017). Confirmed Occurrence of Caligus mugilis Brian (Copepoda, Siphonostomatoida; Caligidae) with Morphological Characters in Turkish Marine Waters. *Thalassas: An International Journal of Marine Sciences*, *33*(2), 133–137. http://doi.org/10.1007/s41208-017-0030-5

Arnal, C., & Côté, I. M. (2000). Diet of broadstripe cleaning gobies on a Barbadian reef. *Journal of Fish Biology*, *57*(4), 1075–1082. http://doi.org/10.1006/jfbi.2000.1371

Arnal, C., Côté, I. M., Sasal, P., & Morand, S. (2000). Cleaner-client interactions on a Caribbean reef: Influence of correlates of parasitism. *Behavioral Ecology and Sociobiology*, *47*(5), 353–358. http://doi.org/10.1007/s002650050676

Arnal, C., & Morand, S. (2001a). Cleaning behaviour in the teleost, Symphodus melanocercus: Females are more specialized that males. *Journal of the Marine Biological Association of the United Kingdom*, *138*(81), 317–323. http://doi.org/10.1017/S0025315401003800

Arnal, C., & Morand, S. (2001b). Importance of ectoparasites and mucus in cleaning interactions in the Mediterranean cleaner wrasse Symphodus melanocercus. *Marine Biology*, *138*(4), 777–784. http://doi.org/10.1007/s002270000494

Arnal, C., Verneau, O., & Desdevises, Y. (2006). Phylogenetic relationships and evolution of cleaning behaviour in the family Labridae: Importance of body colour pattern. *Journal of Evolutionary Biology*, *19*(3), 755–763. http://doi.org/10.1111/j.1420-9101.2005.01059.x

Baliga, V. B., & Law, C. J. (2016). Cleaners among wrasses: Phylogenetics and evolutionary patterns of cleaning behavior within Labridae. *Molecular Phylogenetics and Evolution*, *94*, 424–435. http://doi.org/10.1016/j.ympev.2015.09.006

Barbu, L., Guinand, C., Bergmüller, R., Alvarez, N., & Bshary, R. (2011). Cleaning wrasse species vary with respect to dependency on the mutualism and behavioural adaptations in interactions. *Animal Behaviour*, *82*(5), 1067–1074. http://doi.org/10.1016/j.anbehav.2011.07.043

Bertoncini, Á. A., Machado, L. F., Barreiros, J. P., Hostim-Silva, M., & Verani, J. R. (2009). Cleaning activity among Labridae in the Azores: the rainbow wrasse Coris julis and the Azorean blue wrasse Centrolabrus caeruleus. *Journal of the Marine Biological Association of the United Kingdom*, *89*(4), 859. http://doi.org/10.1017/S002531540900040X

Bshary, R. (2002). Biting cleaner fish use altruism to deceive image-scoring client reef fish. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1505), 2087–2093. http://doi.org/10.1098/rspb.2002.2084

Bshary, R. (2003). The cleaner wrasse, Labroides dimidiatus, is a key organism for reef fish diversity at Ras Mohammed National Park, Egypt. *Journal of Animal Ecology*, *72*(1), 169–176. http://doi.org/10.1046/j.1365-2656.2003.00683.x

Bshary, R., & Grutter, A. S. (2005). Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biology Letters*, *1*(4), 396–399. http://doi.org/10.1098/rsbl.2005.0344

Bshary, R., & Schäffer, D. (2002). Choosy reef fish select cleaner fish that provide high-quality service. *Animal Behaviour*, *63*(3), 557–564. http://doi.org/DOI: 10.1006/anbe.2001.1923

Cheney, K. L., & Côté, I. M. (2001). Are Caribbean cleaning symbioses mutualistic? Costs and benefits of visiting cleaning stations to longfin damselfish. *Animal Behaviour*, *62*(5), 927–933. http://doi.org/10.1006/anbe.2001.1832

Cheney, K. L., & Côté, I. M. (2005). Mutualism or parasitism? The variable outcome of cleaning symbioses. *Biology Letters*, *1*, 162–165. http://doi.org/10.1098/rsbl.2004.0288

Dias, M., Roma, J., Fonseca, C., Pinto, M., Cabral, H. N., Silva, A., & Vinagre, C. (2016). Intertidal pools as alternative nursery habitats for coastal fishes. *Marine Biology Research*, *1000*(April), 1–14. http://doi.org/10.1080/17451000.2016.1143106

Figueiredo, M., Morato, T., Barreiros, J. P., Afonso, P., & Santos, R. S. (2005). Feeding ecology of the white seabream, Diplodus sargus, and the ballan wrasse, Labrus bergylta, in the Azores. *Fisheries Research*, *75*(1–3), 107–119. http://doi.org/10.1016/j.fishres.2005.04.013

Floeter, S. R., Vázquez, D. P., & Grutter, A. S. (2007). The macroecology of marine cleaning mutualisms. *Journal of Animal Ecology*, *76*(1), 105–111. http://doi.org/10.1111/j.1365-2656.2006.01178.x

Galeote, M. D., & Otero, J. G. (1998). 11-Cleaning behaviour of rock cook, Centrolarbrus exoletus (Labridae), in Tarifa (Gibraltar strait area). *Cybium*, *22*(1), 57–68.

Gordoa, A., & Molí, B. (1997). Age and growth of the sparids Diplodus vulgaris, D. sargus and D. annularis in adult populations and the differences in their juvenile growth patterns in the north-western Mediterranean Sea. *Fisheries Research*, *33*(1–3), 123–129. http://doi.org/10.1016/S0165-7836(97)00074-X

Grutter, A. S. (1995). Relationship between cleaning rates and ectoparasite loads in coral reef fishes. *Marine Ecology Progress Series*, *118*(1–3), 51–58. http://doi.org/10.3354/meps118051

Grutter, A. S. (1996a). Experimental demonstration of no effect by the cleaner wrasse Labroides

dimidiatus (Cuvier and Valenciennes) on the host fish Pomacentrus moluccensis (Bleeker). *Journal of Experimental Marine Biology and Ecology*, *196*(1–2), 285–298. http://doi.org/10.1016/0022-0981(95)00135-2

Grutter, A. S. (1996b). Parasite removal rates by the cleaner wrasse Labroides dimidiatus. *Marine Ecology Progress Series*, *130*(1–3), 61–70. http://doi.org/10.3354/meps130061

Grutter, A. S. (1997). Spatiotemporal Variation and Feeding Selectivity in the Diet of the Cleaner Fish Labroides dimidiatus Author (s): Alexandra S . Grutter Spatiotemporal Variation and Feeding Selectivity in the Diet of the Cleaner Fish Labroides dimidiatus. *Copeia*, *2*, 346–355.

Grutter, A. S. (1999). Cleaner fish really do clean. *Nature*, *398*(APRIL), 672–673.

Grutter, A. S., & Bshary, R. (2003). Cleaner wrasse prefer client mucus: support for partner control mechanisms in cleaning interactions. *Proceedings of the Royal Society B*, *270 Suppl*, S242–S244. http://doi.org/10.1098/rsbl.2003.0077

Henriques, M., & Almada, V. C. (1997). Cleaning behaviour in wrasse Arabida.pdf. *Journal of the Marine Biological Association of the United Kingdom*, *77*, 891–898.

Hobson, E. S. (1968). Comments on Certain Recent Generalizations Regarding Cleaning Symbiosis in Fishes. *Pacific Science*, *23*, 35–39.

Johnson, W. S., & Ruben, P. (1988). Cleaning behavior of Bodianus rufus, Thalassoma bifasciatum, Gobiosoma evelynae, and Periclimenes pedersoni along a depth gradient at Salt River Submarine Canyon, St. Croix. *Environmental Biology of Fishes*, *23*(3), 225–232. http://doi.org/10.1007/BF00004913

Krajewski, J. P. (2007). Cleaning by the occasional cleaner Diplodus argenteus (Perciformes: Sparidae) in south Brazil: why so few client species? *Journal of the Marine Biological Association of the UK*, *87*(4), 1013. http://doi.org/10.1017/S0025315407055981

Leitão, F., Santos, M. N., & Monteiro, C. C. (2007). Contribution of artificial reefs to the diet of the white sea bream (Diplodus sargus). *ICES Journal of Marine Science*, *64*(3), 473–478. http://doi.org/10.1093/icesjms/fsm027

Limbaugh, C. (1961). Cleaning symbiosis. *Scientific American*, *205*(2), 42–49. http://doi.org/10.1038/scientificamerican0861-42

Mariani, S. (2001). Cleaning behaviour in Diplodus spp.: chance or choice? A hint for future investigations. *Journal of the Marine Biological Association of the United Kingdom*, *81*(4), 715–716. http://doi.org/017/S0025315401004490

Narvaez, P., Furtado, M., Neto, A. I., Moniz, I., Azevedo, J. M. N., & Soares, M. C. (2015). Temperate facultative cleaner wrasses selectively remove ectoparasites from their client-fish in the Azores. *Marine Ecology Progress Series*, *540*, 217–226.

http://doi.org/10.3354/meps11522

Oates, J., Manica, A., Bshary, R., & Grutter, A. S. (2012). Relationship between roving behaviour and the diet and client composition of the cleaner fish Labroides bicolor. *Journal of Fish Biology*, *81*(1), 210–219. http://doi.org/10.1111/j.1095-8649.2012.03330.x

Pinto, A., Oates, J., Grutter, A. S., & Bshary, R. (2011). Cleaner wrasses labroides dimidiatus are more cooperative in the presence of an audience. *Current Biology*, *21*(13), 1140–1144. http://doi.org/10.1016/j.cub.2011.05.021

Potts, G. W. (1973). Cleaning symbiosis among British fish with special reference to *Crenilabrus melops* (Labridae). *Journal of the Marine Biological Association of the United Kingdom*, *53*(1), 1. http://doi.org/10.1017/S0025315400056587

Reay, P. J., & Cornell, V. (1988). Identification of grey mullet (Teleostei: Mugilidae) juveniles from British waters. *Journal of Fish Biology*, *32*(1), 95–99. http://doi.org/10.1111/j.1095-8649.1988.tb05338.x

Rosecchi, E. (1987). L â€™ Alimentation De Diplodus Annularis , Diplodus Sargus ,. *Revue Des Travaux de l'Institut Des Pêches Maritimes*, *49*(3–4), 125–141.

Sabatino, D., Mori, M., & Tunesi, L. (2007). Interazioni simbiotiche tra il labride pulitore Symphodus melanocercus e i suoi ospitti nel Mar Ligure. *Thalassia Salentia*, *30*, 117–127.

Sala, E., & Ballesteros, E. (1997). Partitioning of space and food resources by three fish of the genus Diplodus (Sparidae) in a Mediterranean rocky infralittoral ecosystem. *Marine Ecology Progress Series*, *152*(1–3), 273–283. http://doi.org/10.3354/meps152273

Sazima, I., Sazima, C., Francini-Filho, R. B., & Moura, R. L. (2000). Daily cleaning activity and diversity of clients of the barber goby, Elacatinus figaro, on rocky reefs in southeastern Brazil. *Environmental Biology of Fishes*, *59*(1), 69–77. http://doi.org/10.1023/A:1007655819374

Soares, M. C., Côté, I. M., Cardoso, S. C., & Bshary, R. (2008). The cleaning goby mutualism: A system without punishment, partner switching or tactile stimulation. *Journal of Zoology*, *276*(3), 306–312. http://doi.org/10.1111/j.1469-7998.2008.00489.x

Stummer, L. E., Weller, J. A., Johnson, M. L., & Côté, I. M. (2004). Size and stripes: How fish clients recognize cleaners. *Animal Behaviour*, *68*(1), 145–150. http://doi.org/10.1016/j.anbehav.2003.10.018

Van Tassell, J. L., Brito, A., & Bortone, S. A. (1994). Cleaning Behavior among marine fishes and invertebrates in the Canary Islands. *Cybium*.

Weitzmann, B., & Mercader, L. (2012). First report of cleaning activity of Lepadogaster candolii (Gobiesocidae) in the Mediterranean Sea. *Cybium*, *36*(3), 487–488.

Whiteman, E., & Côté, I. M. (2002). Cleaning activity of two Caribbean cleaning gobies: intra- and interspecific comparisons. *Journal of Fish Biology*, *60*(6), 1443–1458. http://doi.org/10.1006/jfbi.2002.1947

Zander, C. D., & Sötje, I. (2002). Seasonal and geographical differences in cleaner fish activity in the Mediterranean Sea. *Helgoland Marine Research*, *55*(4), 232–241. http://doi.org/10.1007/s101520100084

6. Appendices

Appendix I

State of the art

Cleaning symbiosis

Natural ecosystems are defined by the abiotic factors and the species that compose them, and are shaped by the nature of the interactions between these composing elements. Within any given ecosystem, each particular species offers different interactions with several other sympatric species, and each of these interspecific interactions possesses specific costs and benefits which might advantage or hinder one or both of the engaging parties.

Typically, interspecific interactions have been classified accordingly to their outcome, meaning the change the interaction has on the overall fitness of each individual, regardless of the scale of this impact. As a result the effects are classified as being negative, positive or neutral, and so there are six possible outcomes we can categorize under this definition, and all six types of interspecies interactions can be observed focusing only on animal species (Holland & DeAngelis, 2009).

Neutralism refers to a situation where there is neither loss nor gain for neither of the interacting individuals, meaning that the interaction has hypothetically no effect. However it is defended by some ecologist that such interactions are impossible, seeing as every action has a given cost, however small it might be, and therefore models of such interactions seem to be only theoretical.

Competition refers to a situation where the interaction shows costs for both implicated organisms. One example of such an interaction occurs when in the same ecosystems several species occupy the same ecological niche, with several overlaps regarding their use of resources and habitat. The availability of resources will change accordingly to the population of these different species, and so, each species will adversely affect the others.

Amensalism represents a situation where one of the involved individuals causes harm to another without any benefit or cost for itself. The best example of these interactions can be seen in accidental encounters between large animals and smaller organisms, while the large animal may be completely unaware of the presence of the smaller being it may cause it accidental arm.

Commensalism represents a situation where one of the involved individuals has neither gain nor loss whereas the other one takes profit from the interaction. There are several examples

of such interactions, like necrophagous feeding where an animal gains a food source through the effort of another, or animals using discarded structures left by other animals.

Exploitation refers to any given interaction where one organism causes direct harm to another for its own benefit, the "winner" usually being dependent of such interaction. The most typical examples of exploitation are predation and parasitism, where one animal feeds upon another, the difference being that predation implies death of the "exploited" while parasitism not necessarily.

Mutualism refers to any given interaction where both of the individuals are benefited from it, implying some sort of two sided collaboration. While there are various and diverse examples of such interactions the one we will further explore is cleaning mutualism.

Cleaning mutualism also called cleaning symbiosis is defined as an interspecies interaction within which an individual defined as cleaner removes ectoparasites, bacteria, dead tissue and/or food particles from another individual defined as client (Hobson, 1968; Poulin & Vickery, 1995; Floeter *et al.*, 2007; Holland & DeAngelis, 2009). The trade off represents a food income for the cleaner, and for the client the removal of potential health hazards. These interactions usually associate a small cleaner species to a much larger client, and cleaners can be found in various ecosystems, and within diverse taxa (Floeter *et al.*, 2007). Such as with other types of symbiosis, cleaning symbiosis can be described as either obligatory or facultative, depending respectively on whether the cleaner depends of this interaction as it represents its principal way of obtaining food, or is only an optional method of food foraging (Arnal & Côté, 2000) . Furthermore while obligatory cleaners will perform such interactions during their entire lifespan, facultative cleaners usually only engage in cleaning activities during their earlier life stages (Narvaez *et al.*, 2015).

In terrestrial ecosystems, the first known description of cleaning behaviour is attributed to an ancient Greek historian named Herodotus during the 5th century before Christ. Herodotus observed a small bird cleaning the inside of a crocodile's mouth in the shore of the Nile River. He reported the encounter giving special emphasis on the lack of aggression the crocodile showed towards the potential prey while allowing it to peck inside its mouth to remove "leeches". Although observations showed that several birds seem to serve as potential occasional cleaners for crocodiles, to date only sandpipers (*Actitis hypoleucos*) have been reported has removing parasites from inside crocodiles' mouth (Cott, 1961).

Several other birds have since then been documented as having similar relations with other animals, to name a few: Darwin finches, who serve as cleaners to Galápagos tortoises and

marine iguanas, the wattled jacana (*Jacana jacana)* has been observed cleaning capybaras (Marcus, 1985), and both oxpeckers species of the *Buphagus* genus are known and even named from the fact they remove ectoparasites from large mammalian herbivores and are often found performing such behaviour (Weeks, 1999). In each of these cases the client seems to allow the bird to remain on its back without attempting to shake it off during the process.

In mammals some isolated episodes of interspecific cleaning are described for different species especially within primates. However due to the mostly social aspect of grooming within mammal species it should be mostly considered that the trade-off of such interactions is socialization. It is therefore more likely that such interactions should be considered as interspecific social grooming and not as specialized cleaning interactions, let alone cleaning symbiosis (Heymann, 1990).

Although these interactions might seem behaviourally complex, they can also be found within invertebrates. Terrestrial pseudoscropions, which are only a few millimetres in size, feed on small arthropods, such as mites and lice, removing them from other arthropods or medium sized mammals. Although this interaction may lack in complexity, with an absence of interspecific communication, due to the fact that the client is mostly unaware of the presence of the cleaner, some pseudoscorpion species have shown to have a crucial role in the health of their respective clients, such as *Chelifer cancroides* who served as an important natural controller of the bee louse in Europe (Donovan & Paul, 2005).

While only first described in the second half of the 19th Century, cleaning mutualism in aquatic and marine ecosystems appears to be more typical than in land. Both facultative and obligatory marine cleaner species appear to be more numerous than terrestrial species, and although initially reported as episodic events, relations of cleaning symbiosis are considered a regular occurrence with ecological relevance within the marine environment(Limbaugh, 1961). As of now cleaning interactions have already been reported for over 110 fish species of 29 different families and 20 invertebrate species from 4 families (Van Tassell *et al.*, 1994; Floeter *et al.*, 2007; Baliga & Law, 2016). Most of these known species can be found within the tropics, although it is argued by some authors that this difference in number might only be a reflection of overall difference in species diversity between tropical and temperate oceans, further underlined by the difference of total observations made between the two environments, as observations in cold and temperate waters are still limited (Limbaugh, 1961; Hobson, 1968; Van Tassell *et al.*, 1994; Sazima *et al.*, 2000).

Within fish species, the two families that seem to possess the larger number of cleaners among them are the Labridae and Gobiidae (Arnal *et al.*, 2006), two families of cosmopolitan distribution (with the exception of the cold polar waters). Several species of Labridae are considered the main cleaners in several biomes such as *Labdroides dimidiatus* in the Indo-Pacific (Grutter, 1996b), *Centrolabrus exoletus* in the North-Eastern Atlantic (Henriques & Almada, 1997) and *Symphodus melanocercus* in the Mediterranean (Zander & Sötje, 2002; Sabatino *et al.*, 2007).

Regardless of their geographical distribution, several characteristics seem to be shared amongst cleaner fish species in marine ecosystems.

Regarding their physical characteristics just as in the case of terrestrial cleaners, cleaner fish tend to be rather small especially when compared to their respective clients (Stummer *et al.*, 2004 Arnal *et al.*, 2006). Many species have also a characteristic coloured pattern, with horizontal stripes of strongly contrasting colours, a pattern that is also found within cleaner shrimp (Potts, 1973; Stummer *et al.*, 2004 Arnal *et al.*, 2006). It is believed that this conspicuous colour motif helps clients recognize cleaners, therefore serving as a visual signal which facilitates interactions. This seems to be further proved by the fact that some facultative cleaners who do not possess such colour characteristics appear to have lower success rates during their interactions (Krajewski, 2007). In the Labridae family there seems to be a close correlation between cleaning behaviour and the existence of such patterns when looking at the phylogeny of the family.

Regarding behaviour, many obligatory cleaners have sedentary territorial life-styles, roaming very little from within a limited area which they might share, depending of the species, with a partner or with hundreds of their conspecific (Hobson, 1968). This territory, designated as cleaning station, is recognized and sought out by potential clients who enter the cleaners' territory seeking out the interaction (Johnson & Ruben, 1988; Cheney & Côté, 2005). The previously mentioned physical and behavioural characteristics of cleaner fish result in a very ritualized and conspicuous procedure during these interactions.

Generally, a potential client will enter the cleaning station, and give the cleaner a visual cue indicating that he is ready to be cleaned. This visual signal usually comes as a specific body posture most notably a head up or head down positioning, meaning the client shifts his body at a 45º to 90 angle upwards or downwards (Galeote & Otero, 1998). This body posture is sometimes also followed by an alteration of the clients body colour. Optionally, the interaction may also be started by the cleaner who directly approaches the potential client without any previous display (Galeote & Otero, 1998).

The cleaner (or cleaners) will then proceed to make a visual inspection of the client's body (Arnal *et al.*, 2000), and choose to either ignore him or proceed with the interaction. Starting with

the first physical contact, the cleaning event may last as the cleaner carries on nipping the client in an interaction that might take from a few seconds up to several minutes (Johnson & Ruben, 1988; Floeter *et al.*, 2007). The interaction ends with either one, or both of the fishes swimming away from the other. It is relevant to note however that there is yet no existing consensus as to what determines the beginning moment of a cleaning event. Some authors choose to name the entire process, starting with visual inspection, following with physical contact and ending with separation as "inspection" (Grutter, 1996b), while others separate visual inspection from the cleaning event, having it commence with the first physical contact between the pair, definition that this work follows.

The number of parasites removed from client fish or frequency of interactions between cleaner and client per unit of time (cleaning rates) seem to vary greatly according to various factors. There is a considerable difference on interaction rates depending of cleaner species (Arnal & Côté, 2000; Narvaez *et al.*, 2015). To compare two species of the Labridae family for instance, *Labroides dimidiatus* has an average of 2297 inspections per day (Grutter, 1996b) while *Coris julis* has an estimated 256 inspections per day (Narvaez *et al.*, 2015). It could be hypothesized that obligatory cleaners, whose feeding greatly depends of such interactions, would have much larger rates of parasite removal than facultative cleaners, however it has already been demonstrated that this rule those not always apply. Grutter & Feeney, 2016 found equivalent client inspection numbers and ectoparasite in gut contents for both juvenile *Diproctacanthus xanthurus* and *Labroides dimidiatus* with the former being a facultative cleaner with only cleaning behaviour in juvenile and the later an obligate cleaner, though both belonging to the Labridae family.

Territorial behaviour of cleaner may also be an important factor as cleaning stations concentrate both cleaners and potential clients within a specific area. Early reports showed that even small cleaning stations could process more than 50 fish per hour (Limbaugh, 1961). Comparing closely related species with different behaviour regarding territory such as *Centrolabrus exoletus* which swims freely and *Symphodus melanocercus* who is solitary and territorial, has underlined this difference, showing that the ability of clients to recognize cleaning locations does facilitate encounters (Galeote & Otero, 1998) resulting in an higher frequency of interactions (Narvaez *et al.*, 2015). Furthermore some cleaning stations may be shared among different cleaner species, which could in turn make them more attractive to clients (Johnson & Ruben, 1988; Quimbayo *et al.*, 2012).

Client species also seems to be important to determine cleaning rates as cleaners do not interact equally with every potential client and seem to actively choose which clients to clean (Sazima *et al.*, 2000). This is underlined by the fact that the most abundant species are not the most frequently cleaned, although this may be true with certain cleaners (Galeote & Otero, 1998). While potential predators are more actively avoided and have shorter interactions (Oates *et al.*, 2012), bigger fish seem to be preferred by cleaners (Arnal *et al.*, 2000). This preference of bigger clients may be due to the high correlation size has with parasitic load, with fishes with higher body mass and surface area being more prone to ectoparasites. Cleaners have shown to prioritize and take more time inspecting and interacting with bigger clients, which happens not only between species of different sizes, but also with different size classes within the same species (Grutter, 1995; Arnal *et al.*, 2000).

Furthermore has there appears to be selectivity regarding ingested ectoparasites by cleaners (Grutter, 1997; Narvaez *et al.*, 2015) the life-cycle of these parasites may alter cleaning frequency. First due to parasites not being equally present throughout the year, specially within temperate waters (Zander & Sötje, 2002), cleaning rates may vary accordingly (Cheney & Côté, 2005; Grutter, 1997). Ectoparasites may also have a preference for different hosts, which may also be one of the reason cleaners are selective about their clients.

Cleaning symbiosis: parasitism, commensalism or mutualism?

The greatest question regarding cleaning symbiosis remains, is it truly a mutualistic relationship? The question arises mostly from the difficulty to quantify hypothetical costs and benefits within the interaction, especially concerning client species (Cheney & Côté, 2005). While the benefit for the cleaner is apparent, and could be quantified by calculating ingested biomass, the benefit for the cleaner remains hard to appraise for several reasons. It is nearly impossible to assess the payoff of having parasites removed, as it is hard to truly understand the impact cleaning activity as on overall parasite load (Grutter, 1996b).

While benefits are difficult to assess, cleaning interactions for clients, represents an investment in time, that not only sacrifices periods that could be used for foraging or other activities, but also, represents potentially a rise in the risk of predation for roving fish as they might swim through more dangerous areas to reach cleaning stations (Cheney & Côté, 2005; Oates *et al.*, 2012). Furthermore as with many interspecific or intraspecific interactions, there are incidents of cheating. In the case of cleaning interactions, while cheating from the client

perspective means cases of predation of the cleaner, which are rare, cases of cheating from the cleaner are defined as episodes of parasitism (Poulin & Vickery, 1995), where cleaners bite of scales and mucus from the clients. The ectodermic mucus in fish serves as a protective lair that may in some cases even have some anti-parasitic properties and is rich in proteins. The removal of this mucus by cleaner fish represents therefore not only a direct harm to the client, but more ironically, might actually increase the risk of infection in the bitten area. Some studies have proven that it is more rewarding for cleaner fish to bite off rich mucus than to feed solely in parasites, and it is known that some cleaner fish actively cheat for their own gain (Arnal & Morand, 2001; Grutter & Bshary, 2003). The "cheating rate" by cleaners seems to vary according to cleaner species (Soares *et al.*, 2008) and client species (R. Bshary, 2002). Ultimately this preference for mucus could also be one of the reasons behind client selectivity observed during interactions (Arnal & Morand, 2001).

Another case that furthers demonstrates potential cheating from cleaner fish comes as a behaviour demonstrated by some of the most specialized cleaners, tactile stimulation. This behaviour consists in cleaners giving small touches with their pectoral fins to give small caresses to the client, and this tactile stimulation is physically rewarding to the client. *Labroides dimidiatus,* is one of the cleaners known to perform such behaviour and clients seem to prefer cleaners that perform good tactile stimulation which ultimately can reduce their response to stress (Bshary *et al.*, 2007). This manipulation could therefore be a mechanism to allow cleaners higher degrees of cheating without suffering trade off from the clients.

Clients seem to possess some mechanisms to reduce potential cheating from cleaners, namely, punishment and partner switching. Clients which are cheated on by cleaners will often end the interaction, usually shaking off the cleaner in a movement usually described as a "jolt". Being a conspicuous behaviour, and having a high degree of correlation, jolts are usually used as a measurement of cheating (R. Bshary & Schäffer, 2002; Barbu *et al.*, 2011). These jolts are also sometimes followed by chasing off the cleaner (R. Bshary & Grutter, 2005). Some clients will also accordingly to the outcome of the interaction show a higher probability of returning to the same cleaning station accordingly to whether or not there was cheating or they saw cleaners cheat with their previous clients. This could ultimately reduce cheating events as non-cheaters would be awarded with a more steady income of clients.

The "cheating rate" performed by cleaners seems to vary accordingly however. In a work by Pinto *et al* 2011, it was found that *Labroides dimidiatus* were sensible to the audience effect, showing higher cooperation with their clients when being on the presence of other fish. In other hand, cheating rates appear to be higher with non territorial client and cleaner species and lower

with territorial species with which interactions may be recurrent (Oates et al., 2012). This ultimately proves a species dependant outcome and a conflict of interest between clients and cleaners.

In opposition to the situation mentioned above, Soares *et al.*, 2008 reported within *Elecatinus* sp. the absence of client control mechanisms. While these cleaner gobies do not perform tactile stimulation of their clients, the clients return rate seemed to have no link with the outcome of previous interactions. Three hypothesis were discussed to the absence of these client control mechanism: 1) Constraints: either by cleaners or by clients being less dependent of these interactions, or clients having cognitive constraints being therefore unable to develop the ability to punish cleaners; 2) Low cost of being cheated: meaning no fitness costs on clients when cheated; 3) Foraging preference hypothesis: *Elecatinus* could be less interested in mucus and therefore naturally more honest which could make cheating rare and ultimately negligible.

Ultimately, to prove the impact of cleaners on the life quality of clients and their importance within ecosystems, cleaner removal has been tested in several studies. While Limbaugh, 1961 reported that within 2 weeks after the removal of all cleaner species, almost all non territorial fish had disappeared from the reef, it failed to report quantitative data. Grutter, 1996a attempted to quantify the impact of cleaner *Labroides dimidiatus* through mean number of parasites in client species *Pomacentrus moluccensis*. As cleaners usually are only active during daylight hours (Johnson & Ruben, 1988; Grutter, 1996b; Sazima *et al.*, 2000) while parasites usually attach to host at night, the parasite count should show the impact cleaning had with parasite load after removal of the cleaner. The results however should no difference in mean number of parasites or average size of parasites within test location and control, meaning that at least for this client species, there should be no difference in mortality reported. It was however underlined that *Labroides dimidiatus* tend to prefer large gnathiids as parasites, while *Pomacentrus moluccensis* had no gnathiids and only small parasites. Grutter, 1999 further developed on this point by studying another client species *Hemigymmus melapterus* affected mainly by gnathiids. In this two part experiment that accounted for the difference in parasites within 12h and within 12 days, the sites where *Labroides dimidiatus* had been removed showed respectively a 4.5 and a 3.8 times increase in gnathiid when compared to control. Although the impact on fish of these parasite loads is hard to quantify, and therefore hard to know if there is a real difference in mortality between more and less infected individuals, the study did show that ultimately the cleaners did have an impact in parasite load within a short time period. R. Bshary, 2003 attempted an experiment in a period up to 20 months In this case, the aim of the study was to verify how would the disappearance of

Labroides dimidiatus affect the fish diversity, such has what was observed in Limbaugh, 1961. In the experiment they tried both addition of cleaners in reefs where they were absent and removal of cleaners in sites where they were present. Higher diversity of species in reefs where *Labroides dimidiatus* was added was observed after 2-4 weeks, while reefs where *Labroides dimidiatus* was removed only had a decrease in species diversity observed after 4-20 months. The differences were also higher in visiting clients, although these represented only 9-29% of total species diversity, and lower in non-client territorial species. It was hypothesized that cleaners are especially relevant during habitat choice for other species, which will favourite reefs where cleaners are present. Cheney & Côté, 2005 attempted an experiment similar to Grutter, 1999 focusing in independent sites along the Caribbean with the cleaners *Elacatinus evelynae* and client *Stegastes diencaeus*. In this experiment, results showed that cleaners did impact ectoparasite loads in sites where clients were highly infected, but had no impact in regions where ectoparasite loads were low. Gut contents also showed that in the same locations where ectoparasite loads did not change with cleaner presence or absence, the proportion of mucus and client scales were higher. Ultimately, this shows that these interactions can be either mutualistic or parasitic depending on the availability of parasite which may promote cleaners honesty.

It may therefore be summarized that the outcome of cleaning symbiosis is dependant of several factors; such as parasite species, parasite-load, cleaner species and client species. While feeding solely on parasites may be a rewarding enough food-source and client punishment may encourage honesty within cleaners, cleaners may eventually in situations show opportunistic cheating behaviour and feed on client scales and mucus, making cleaning symbiosis parasitic. Within certain ecosystems, cleaning symbiosis plays an important role, not only helping reducing ectoparasite loads within client species, but also, serving as a determinant factor for species immigration within the area. As such cleaning symbiosis should most probably be considered a mutualistic behaviour with a high cheating occurrence.

Cleaners within the Sparidae family

While cleaning species have been meticulously studied within the Labridae and Gobidae families, especially within the tropical species where interaction frequency, client species and cleaning patterns of cleaners have already been reported, cleaning behaviour within species of other families is still poorly understood.

The Sparidae are a good example to underline this lack of understanding. While cleaning behaviour has been observed and reported within the family (Moosleitner, 1980), there are no

descriptions of this behaviour in the literature. The presence of ectoparasites in gut contents of *Diplodus sargus* (Rosecchi, 1987)and *Diplodus puntazzo* (Mariani, 2001) reinforced the believe that cleaning behaviour existed within the genus, but no observations were made of the behaviour.

References

Arnal, C., & Côté, I. M. (2000). Diet of broadstripe cleaning gobies on a Barbadian reef. *Journal of Fish Biology*, *57*(4), 1075–1082. http://doi.org/10.1006/jfbi.2000.1371

Arnal, C., Côté, I. M., Sasal, P., & Morand, S. (2000). Cleaner-client interactions on a Caribbean reef: Influence of correlates of parasitism. *Behavioral Ecology and Sociobiology*, *47*(5), 353–358. http://doi.org/10.1007/s002650050676

Arnal, C., & Morand, S. (2001). Importance of ectoparasites and mucus in cleaning interactions in the Mediterranean cleaner wrasse Symphodus melanocercus. *Marine Biology*, *138*(4), 777–784. http://doi.org/10.1007/s002270000494

Arnal, C., Verneau, O., & Desdevises, Y. (2006). Phylogenetic relationships and evolution of cleaning behaviour in the family Labridae: Importance of body colour pattern. *Journal of Evolutionary Biology*, *19*(3), 755–763. http://doi.org/10.1111/j.1420-9101.2005.01059.x

Baliga, V. B., & Law, C. J. (2016). Cleaners among wrasses: Phylogenetics and evolutionary patterns of cleaning behavior within Labridae. *Molecular Phylogenetics and Evolution*, *94*, 424–435. http://doi.org/10.1016/j.ympev.2015.09.006

Barbu, L., Guinand, C., Bergmüller, R., Alvarez, N., & Bshary, R. (2011). Cleaning wrasse species vary with respect to dependency on the mutualism and behavioural adaptations in interactions. *Animal Behaviour*, *82*(5), 1067–1074. http://doi.org/10.1016/j.anbehav.2011.07.043

Bshary, R. (2002). Biting cleaner fish use altruism to deceive image-scoring client reef fish. *Proceedings of the Royal Society B: Biological Sciences*, *269*(1505), 2087–2093. http://doi.org/10.1098/rspb.2002.2084

Bshary, R. (2003). The cleaner wrasse, Labroides dimidiatus, is a key organism for reef fish diversity at Ras Mohammed National Park, Egypt. *Journal of Animal Ecology*, *72*(1), 169–176. http://doi.org/10.1046/j.1365-2656.2003.00683.x

Bshary, R., & Grutter, A. S. (2005). Punishment and partner switching cause cooperative behaviour in a cleaning mutualism. *Biology Letters*, *1*(4), 396–399. http://doi.org/10.1098/rsbl.2005.0344

Bshary, R., Oliveira, R. F., Oliveira, T. S., & Canário, A. V. (2007). Do cleaning organisms reduce the stress response of client reef fish? *Frontiers in Zoology*, *4*(1), 21. http://doi.org/10.1186/1742-9994-4-21

Bshary, R., & Schäffer, D. (2002). Choosy reef fish select cleaner fish that provide high-quality service. *Animal Behaviour*, *63*(3), 557–564. http://doi.org/DOI: 10.1006/anbe.2001.1923

Cheney, K. L., & Côté, I. M. (2005). Mutualism or parasitism? The variable outcome of cleaning symbioses. *Biology Letters*, *1*, 162–165. http://doi.org/10.1098/rsbl.2004.0288

Cott, H. B. (1961). Scientific results into of an inquziry into the ecology and economic status of the Nile crocodile in In Uganda and Northern Rhodesia. *Trans. Zool. Soc. London*, *29*, 211–356.

Donovan, B. J., & Paul, F. (2005). Pseudoscorpions: The forgotten beneficials inside beehives and their potential for management for control of varroa and other arthropod pests. *Bee World*, *86*(4), 83–87. http://doi.org/10.1080/0005772X.2005.11417322

Floeter, S. R., Vázquez, D. P., & Grutter, A. S. (2007). The macroecology of marine cleaning mutualisms. *Journal of Animal Ecology*, *76*(1), 105–111. http://doi.org/10.1111/j.1365-2656.2006.01178.x

Galeote, M. D., & Otero, J. G. (1998). 11-Cleaning behaviour of rock cook, Centrolarbrus exoletus (Labridae), in Tarifa (Gibraltar strait area). *Cybium*, *22*(1), 57–68.

Grutter, A. S. (1995). Relationship between cleaning rates and ectoparasite loads in coral reef fishes. *Marine Ecology Progress Series*, *118*(1–3), 51–58. http://doi.org/10.3354/meps118051

Grutter, A. S. (1996a). Experimental demonstration of no effect by the cleaner wrasse Labroides dimidiatus (Cuvier and Valenciennes) on the host fish Pomacentrus moluccensis (Bleeker). *Journal of Experimental Marine Biology and Ecology*, *196*(1–2), 285–298. http://doi.org/10.1016/0022-0981(95)00135-2

Grutter, A. S. (1996b). Parasite removal rates by the cleaner wrasse Labroides dimidiatus. *Marine Ecology Progress Series*, *130*(1–3), 61–70. http://doi.org/10.3354/meps130061

Grutter, A. S. (1997). Spatiotemporal Variation and Feeding Selectivity in the Diet of the Cleaner Fish Labroides dimidiatus Author (s): Alexandra S . Grutter Spatiotemporal Variation and Feeding Selectivity in the Diet of the Cleaner Fish Labroides dimidiatus.

Copeia, *2*, 346–355.

Grutter, A. S. (1999). Cleaner fish really do clean. *Nature*, *398*(APRIL), 672–673.

Grutter, A. S., & Bshary, R. (2003). Cleaner wrasse prefer client mucus: support for partner control mechanisms in cleaning interactions. *Proceedings of the Royal Society B*, *270 Suppl*, S242–S244. http://doi.org/10.1098/rsbl.2003.0077

Grutter, A. S., & Feeney, W. E. (2016). Equivalent cleaning in a juvenile facultative and obligate cleaning wrasse: an insight into the evolution of cleaning in labrids? *Coral Reefs*, *35*(3), 991–997. http://doi.org/10.1007/s00338-016-1460-x

Henriques, M., & Almada, V. C. (1997). Cleaning behaviour in wrasse Arabida.pdf. *Journal of the Marine Biological Association of the United Kingdom*, *77*, 891–898.

Heymann, E. W. (1990). lnterspecific Relations in a Mixed-Species Troop of SaddIe-Back Tamarins, Saguinus fuscicollis (Platyrrhini: Callitrichidae), at the Rio Blanco, Peruvian Amazonia. *American Journal of Primatology*, *21*, 115–127.

Hobson, E. S. (1968). Comments on Certain Recent Generalizations Regarding Cleaning Symbiosis in Fishes. *Pacific Science*, *23*, 35–39.

Holland, J., & DeAngelis, D. (2009). Consumer-resource theory predicts dynamic transitions between outcomes of interspecific interactions. *Ecology Letters*, *12*, 1357–1366. http://doi.org/10.1111/j.1461-0248.2009.01390.x

Johnson, W. S., & Ruben, P. (1988). Cleaning behavior of Bodianus rufus, Thalassoma bifasciatum, Gobiosoma evelynae, and Periclimenes pedersoni along a depth gradient at Salt River Submarine Canyon, St. Croix. *Environmental Biology of Fishes*, *23*(3), 225–232. http://doi.org/10.1007/BF00004913

Krajewski, J. P. (2007). Cleaning by the occasional cleaner Diplodus argenteus (Perciformes: Sparidae) in south Brazil: why so few client species? *Journal of the Marine Biological Association of the UK*, *87*(4), 1013. http://doi.org/10.1017/S0025315407055981

Limbaugh, C. (1961). Cleaning symbiosis. *Scientific American*, *205*(2), 42–49. http://doi.org/10.1038/scientificamerican0861-42

Marcus, M. J. (1985). Feeding associations between capybaras and jacanas: a case of interspecific grooming and possibly mutualism. *Ibis*, *127*(2), 240–243. http://doi.org/10.1111/j.1474-919X.1985.tb05058.x

Mariani, S. (2001). Cleaning behaviour in Diplodus spp.: chance or choice? A hint for future investigations. *Journal of the Marine Biological Association of the United Kingdom*, *81*(4), 715–716. http://doi.org/017/S0025315401004490

Moosleitner, V. H. (1980). Putzerfische und -garnelen im Mittelmeer. *Zool.Anz., Jena*,

205(3/4), 219–240.

Narvaez, P., Furtado, M., Neto, A. I., Moniz, I., Azevedo, J. M. N., & Soares, M. C. (2015). Temperate facultative cleaner wrasses selectively remove ectoparasites from their client-fish in the Azores. *Marine Ecology Progress Series*, *540*, 217–226. http://doi.org/10.3354/meps11522

Oates, J., Manica, A., Bshary, R., & Grutter, A. S. (2012). Relationship between roving behaviour and the diet and client composition of the cleaner fish Labroides bicolor. *Journal of Fish Biology*, *81*(1), 210–219. http://doi.org/10.1111/j.1095-8649.2012.03330.x

Pinto, A., Oates, J., Grutter, A. S., & Bshary, R. (2011). Cleaner wrasses labroides dimidiatus are more cooperative in the presence of an audience. *Current Biology*, *21*(13), 1140–1144. http://doi.org/10.1016/j.cub.2011.05.021

Potts, G. W. (1973). Cleaning symbiosis among British fish with special reference to *Crenilabrus melops* (Labridae). *Journal of the Marine Biological Association of the United Kingdom*, *53*(1), 1. http://doi.org/10.1017/S0025315400056587

Poulin, & Vickery, W. L. (1995). Cleaning symbiosis as an evolutionary game: To cheat or not to cheat? *Journal of Theoretical Biology*, *175*(1), 63–70. http://doi.org/10.1006/jtbi.1995.0121

Quimbayo, J. P., Floeter, S. R., Noguchi, R., Rangel, C. a., Gasparini, J. L., Sampaio, C. L. S., … Rocha, L. a. (2012). Cleaning mutualism in Santa Luzia (Cape Verde Archipelago) and São Tomé Islands, Tropical Eastern Atlantic. *Marine Biodiversity Records*, *5*(December), e118. http://doi.org/10.1017/S175526721200108X

Rosecchi, E. (1987). L â€™ Alimentation De Diplodus Annularis , Diplodus Sargus ,. *Revue Des Travaux de l'Institut Des Pêches Maritimes*, *49*(3–4), 125–141.

Sabatino, D., Mori, M., & Tunesi, L. (2007). Interazioni simbiotiche tra il labride pulitore Symphodus melanocercus e i suoi ospitti nel Mar Ligure. *Thalassia Salentia*, *30*, 117–127.

Sazima, I., Sazima, C., Francini-Filho, R. B., & Moura, R. L. (2000). Daily cleaning activity and diversity of clients of the barber goby, Elacatinus figaro, on rocky reefs in southeastern Brazil. *Environmental Biology of Fishes*, *59*(1), 69–77. http://doi.org/10.1023/A:1007655819374

Soares, M. C., Côté, I. M., Cardoso, S. C., & Bshary, R. (2008). The cleaning goby mutualism: A system without punishment, partner switching or tactile stimulation. *Journal of Zoology*, *276*(3), 306–312. http://doi.org/10.1111/j.1469-7998.2008.00489.x

Stummer, L. E., Weller, J. A., Johnson, M. L., & Côté, I. M. (2004). Size and stripes: How fish clients recognize cleaners. *Animal Behaviour*, *68*(1), 145–150. http://doi.org/10.1016/j.anbehav.2003.10.018

Van Tassell, J. L., Brito, A., & Bortone, S. A. (1994). Cleaning Behavior among marine fishes and invertebrates in the Canary Islands. *Cybium*.

Weeks, P. (1999). Interactions between red-billed oxpeckers, Buphagus erythrorhynchus, and domestic cattle, Bos taurus, in Zimbabwe. *Animal Behaviour*, *58*(6), 1253–1259. http://doi.org/10.1006/anbe.1999.1265

Zander, C. D., & Sötje, I. (2002). Seasonal and geographical differences in cleaner fish activity in the Mediterranean Sea. *Helgoland Marine Research*, *55*(4), 232–241. http://doi.org/10.1007/s101520100084

Appendix II

Sampling locations

The general layout of each of these harbours and their location along the Portuguese coast is shown bellow through satellite pictures.

Figure a. Top Left- Porto de Póvoa de Varzim, 41º 22' 08" N, 8º 45' 49" W, taken from: https://www.google.pt/maps/place/Marina+da+P%C3%B3voa+de+Varzim/@41.3689088,-8.7657408,17z/data=!3m1!4b1!4m5!3m4!1s0xd2445dbef70038f:0xc11dc4153e3b8a7c!8m2!3d41.3689088!4d-8.7635521 in June 12 2017.

Top Right- Marina de Oeiras - Porto de Recreio, 38º 40' 34" N, 9º 19' 05" W, taken from: https://www.google.pt/maps/place/Marina+de+Oeiras+-+Porto+de+Recreio/@38.6759734,-9.3203117,17z/data=!3m1!4b1!4m5!3m4!1s0xd1ec9194a13e285:0x27c408bf393cc028!8m2!3d38.6759734!4d-9.318123 in June 12 2017.

Bottom- Marina de Tróia, 38º 29' 36" N, 8º 54' 10" W, taken from: https://www.google.pt/maps/search/marina+de+troia/@38.5079415,-8.9153004,4756m/data=!3m1!1e3 in June 12 2017.

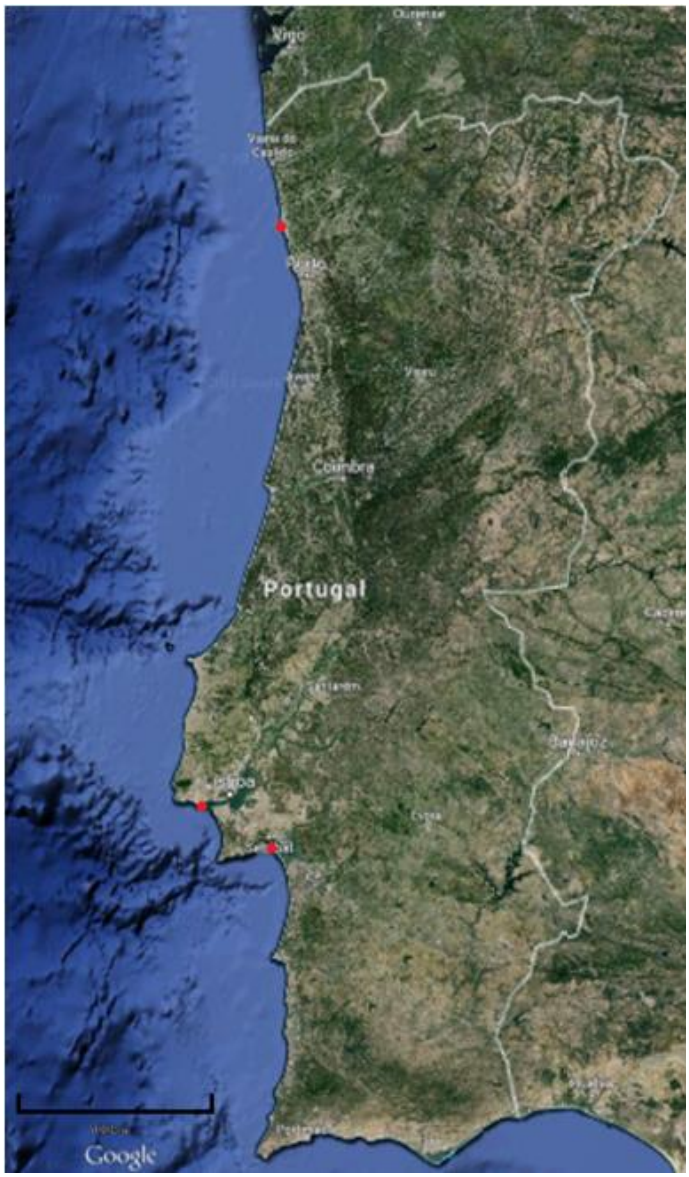

Figure b. Marked locations of the three sampling locations along the Portuguese coast, taken from: https://www.google.pt/maps/place/Portugal/@38.9068176,-8.9880028,619104m/data=!3m1!1e3!4m5!3m4!1s0xb32242dbf4226d5:0x2ab84b091c4ef041!8m2!3d39.399872!4d-8.224454 in June 12 2017.

Appendix III

Morphological identification in the *Diplodus* genus.

Although the various species of the genus *Diplodus* share several physical characteristics, there are some distinctive features among species. Within our study area four species were present, *D. sargus*, *D. vulgaris, D. cervinus* and *D. puntazzo*, while larvae are impossible to identify at the naked eye, individuals that corresponded to our observable minimum (~1cm) already present distinctive features which they mostly maintain as they grow.

As we followed a methodology based only on visual observations and made identification only through morphological features, we present here a summary on how to quickly identify the four *Diplodus* species.

1. *Diplodus sargus*

Evident morphological features: Silver-grey body with pattern of darker transversal stripes along the body starting behind the opercle (5 black stripes alternating with 4 grey stripes) and ending with a dark spot at behind the last rays of the dorsal fin. The dark stripes may be more or less contrasted with some intraspecific variability, in younger juveniles the 5 black stripes are usually more marked while the 4 paler ones are not visible.

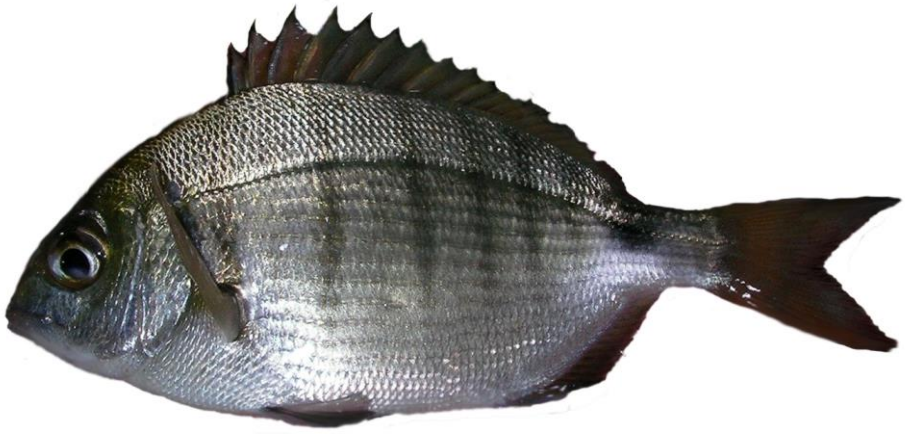

Figure a. *Diplodus sargus*. Picture from personal archive.

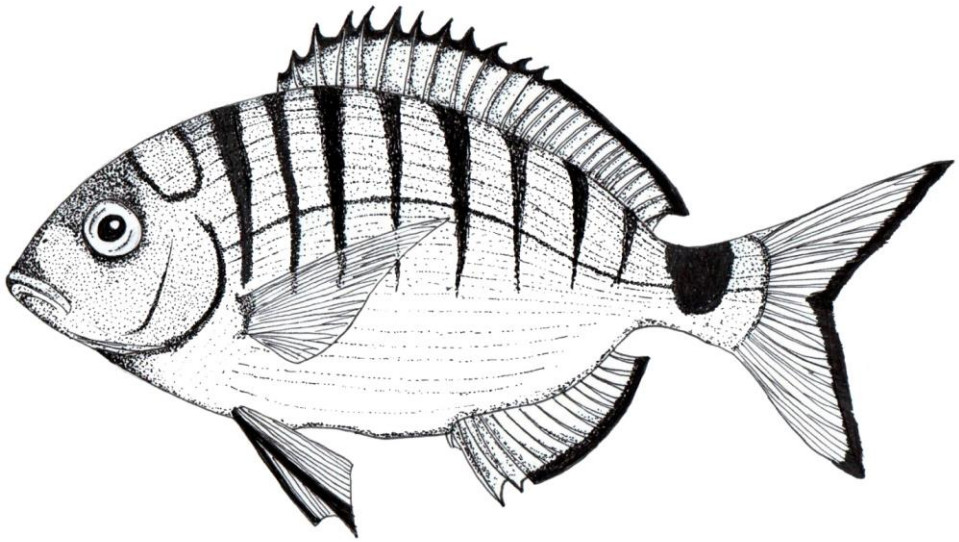

Figure b. Hand-drawn representation of *Diplodus sargus*.

2. *Diplodus cervinus*

Evident morphological features: Silver-grey or brownish-grey body with 5 larger dark transverse bands starting at the base of the pectoral fin, and darker area on the head around the orbit.

Figure c. *Diplodus cervinus*. Picture from personal archive.

3. *Diplodus puntazzo*

Evident morphological features: Silver-grey body with pattern of darker transversal stripes along the body starting behind the opercle alternating between darker and lighter ones, up to 11-13 total and ending with a darker spot behind the dorsal fin (colouration fades with age), head and snout elongated.

Figure d. *Diplodus puntazzo.* Picture from personal archive.

4. *Diplodus vulgaris*

Evident morphological features: Silver-grey with discrete longitudinal golden stripes along the body, two broad black bands, first one starting in front of the dorsal fin and ending near the pectoral axil and second one starting near the base of the last dorsal fin-rays and ending at the base of the anal fin.

Figure e. *Diplodus vulgaris.* Picture from personal archive.